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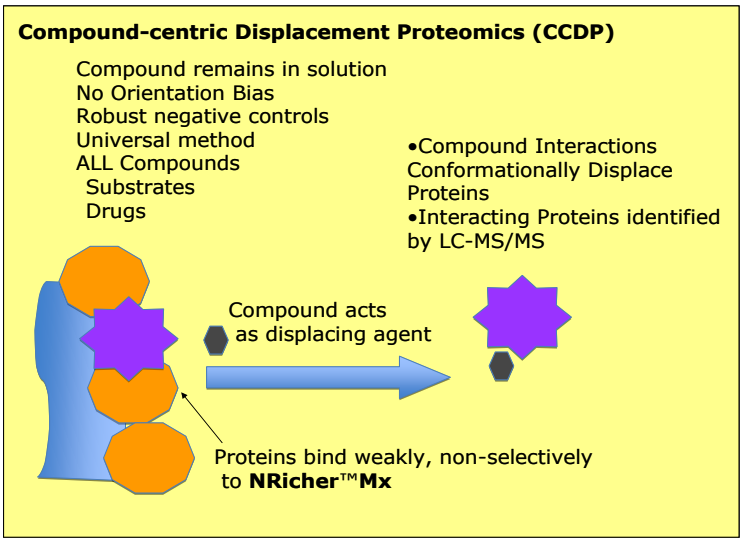
NuGel™ NRicher™ Mx

Compound Centric Displacement Proteomics (CCDP)

- Non-covalently binds proteins, a subset of which can be displaced upon introduction of soluble small compounds
- Coupled to LC-MS, spectral counts of affinity-eluted pools can serve as quantitative metrics to characterize and identify interacting proteins
- Gain efficiencies over prior covalent-based substitution methods: more complete survey, no orientation bias, robust background controls
- Applications in drug target deconvolution, on-target/off-target specificity, and personalized medicine.



MS2 Spectral Counts of CCDP-derived Sub-proteomes

Protein Description	Caffeine	Imatinib	Neg.Cont.
Hemoglobin subunit beta-1	87	550	53
Glucose-6-phosphate isomerase	192	459	76
Malate dehydrogenase	117	356	35
Transketolase	72	160	24
Cytochrome c, somatic	47	123	3
Succinyl-CoA:3-ketoacid transf	69	122	19
Transgelin	0	84	0
Annexin A2	26	66	0
Fumarate hydratase	17	42	2
Annexin A3	5	36	0
Glutathione reductase	9	38	0

A partial list of LC-MS/MS identification and spectral counts demonstrate Imatinib interaction proteins from

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Product	# of preps*	Item No.
NuGel™ NRicher™ Mx	10	SRPRO-10
NuGel™ NRicher™ Mx	50	SRPRO-50

*Each prep processes approximately 0.5-1.0 mg total protein

The **NuGel™ NRicher™ Mx** product kit includes all surface reagents, binding and elution buffers and associated separations protocols. Each prep processes approximately 0.5-1.0 mg total protein, and produces eluate sub-proteomes in 60 µl volumes in less than 1 hour. Efficient low abundance proteome enrichment and protein compression may require higher loads, and will vary with sample type and user requirements.

NuGel™ NRicher™ Mx supports two application protocols:

- **Compound Centric Displacement Proteomics (CCDP) &**
- **Low Abundance Proteome Enrichment**

The protocols are supplied as two separate user documents.

Kit Contains:	NuGel™ NRicher™ Mx 10	NuGel™ NRicher™ Mx 50
NuGel™ NRicher™ Mx beads	150 mg	750 mg
***PRO*-BB** Binding Buffer, pH 6	3 ml	15 ml
PRO-WB Wash Buffer pH 7	5 ml	25 ml
***PRO*-CEB** Elution Buffer, pH 7 (for **Compound Centric Displacement Proteomics Protocol**) only	1 ml	5 ml
***PRO*-EB** Elution Buffer, pH 10 (for **Low Abundance Proteome Enrichment Protocol**) only	1 ml	5 ml
Spin-X microfuge filters	10	50

Compound Centric Displacement Proteomics (CCDP)

Protocol

Step 1 - Sample Preparation: The protocol is based on 100 µl of tissue homogenates with a soluble protein content in the 5 – 15 mg/ml range, per prep. Larger volumes of lower protein content can also be used for load but the total protein content applied should be in the range of 0.5 – 1.5 mg. For serum/plasma, we recommend at least 25 µl be applied.

For best results – the lysate should be clear and free of colloidal material. We recommend first filtering through a 0.45 µm syringe-type filter before beginning the prep.

It has not been evaluated on membrane or insoluble protein content, but it is compatible with up to 0.1% Triton X-100. For best results, pH for samples should be in the range of 6-7.

Step 2 - Surface Preparation. The **NuGel™ NRicher™ Mx** reagent is supplied in dry powder form. **Weigh out 15 mg for each prep and place into the Spin-X filters provided.** Before using, tap each to ensure powders are at the bottom of the filter cup.
1) Add 100 µl of **PRO-BB binding buffer** to each **reagent powder** and mix for 3 minutes.
2) Centrifuge at [5,000-7,000]xg for 4 min. and discard the flow-through.

For Steps 3 & 4. All centrifugations are for 4 minutes at 10,000 rpm.

Step 3 - Separations.
1) Add 100 µl of **PRO-BB binding buffer** and 100 µl (or adjusted volume as stated above) sample, to each prep (from Step 2). Mix until homogeneously resuspended, and shake the mixture for 10 minutes. Centrifuge and discard filtrate.
2) Add 250 µl of **PRO-WB wash buffer** to each surface as a wash. Mix until homogeneously resuspended, and shake the mixture for 10 minutes. Centrifuge and discard filtrate. Repeat wash step 2 again.

Step 4 – Compound-centric displacement elution.
For compound-centric displacement proteomic, any challenging displacement compound can be added into the **PRO-CEB Elution buffer** (subject to solubility constraints) and used as an eluate. Use 60 µl. Mix to homogeneously resuspend. Shake the sample for 10 minutes. Centrifuge. Collect eluate filtrates for analyses.

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Related Functional Proteomic Product - NuGel™ NRicher™ 6

Functional proteomics relies in part, on the functional or structural features of intact, non-denatured proteins. While the terminology can often overlap, chemical and affinity-based proteomic profiles can be considered a subset of functional proteomics. Both **NuGel™ NRicher™ 6** and **NuGel™ NRicher™ Mx** support functional and chemical proteomics and can:

- Optimize drug compounds
- Survey compound promiscuity
- Deconvolute targets, elucidate mechanism of action
- Identify phenotypic biomarkers

Please consult our Functional & Chemical Proteomics Handbook online, to see how NuGel™ NRicher™ Mx and NuGel™ NRicher™ 6 complement each other.

References

Matthew P. Kuruc, Swapan Roy. The Functional & Chemical Proteomics Handbook03/2014

Oka, Amita R., Matthew P. Kuruc, Ketan M. Gujarathi, and Swapan Roy. "Functional Proteomic Profiling of Phosphodiesterases Using SeraFILE Separations Platform." *International Journal of Proteomics* 2012 (2012).

New Chemical Proteomic Methods To Access Drug-Protein Interactions

US HUPO 2014. Frontiers in Proteomics: Advancing Biology through Technology and Computation.
NuGel™ *PRO*fessor™ abstract entitled "Compound-Centric Displacement Proteomics - An Advantaged Method To Survey Small Molecule-Protein Interactions" poster board 096 presented at US HUPO 2014

CONTACT US

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